Evaluation of Subclinical Inflammation with Neutrophil Lymphocyte Ratio In Heavy Metal Exposure

Ağır Metal Maruziyetinde Subklinik Enflamasyonun Nötrofil Lenfosit Oranı ile Değerlendirilmesi

Neutrophil Lymphocyte Ratio in Metal Exposure

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Özet

Amaç: Nötrofil lenfosit oranı (NLR), subklinik enflamasyon belirteci olarak sıklıkla kullanılır. Bu çalışmanın amacı, kurşun maruziyeti olan işçilerde NLR ve ağır metal seviyeleri arasındaki ilişkiyi araştırmaktır. Gereç ve Yöntem: Kurşun maruziyeti olan 1820 bireyin demografik ve laboratuar verileri retrospektif olarak araştırıldı. C reaktif protein (CRP), eritrosit sedimentasyon hızı (ESR) ve tam kan sayımı inflamatuar belirteçler olarak değerlendirildi. Çalışmaya dahil edilen kişiler kan kurşun seviyesi < 10 µg/dL (1. Grup), 10-30 µg/dL (2. Grup), > 30 µg/dL (3. Grup) olacak şekilde 3 gruba ayrıldı. NLR ve kurşun arasındaki ilişki gruplar arası ve korelasyon analizi ile incelendi. Bulgular: 1., 2. ve 3. grubun NLR median değerleri sırasıyla 1. 45 (1.57), 1.90 (6.44) ve 1.96 (6.36) olarak bulundu (p<0.001). NLR kan kurşun seviyeleri ile pozitif olarak korele tespit edildi (r=0.412; p<0.001). NLR ile CRP, ESR, beyaz kan hücresi, nötrofil ve ortalama platelet hacmi seviyeleri arasında da pozitif korelasyon bulundu (sırasıyla, r=0.140; p<0.001, r=0.075; p=0.002, r=0.237; p<0.001, r=0.585; p<0.001, r=0.060; p<0.012). NLR ile lenfosit seviyeleri arasında ise negatif korelasyon elde edildi (r= -0.536; p<0.001). Tartışma: Bu çalışma, bildiğimiz kadarıyla, NLRve kurşun seviyeleri arasında güçlü ve doz bağımlı ilişkiyi gösteren ilk araştırmadır.

Anahtar Kelimeler

Kurşun; Nötrofil; Lenfosit; Enflamasyon

Abstract

Aim: Neutrophil lymphocyte ratio (NLR) is being used frequently as a marker of subclinical inflammation. The objective of this study is to investigate the association between NLR levels and heavy metal levels in workers with lead exposure. Material and Method: Demographic and laboratory data of 1820 individuals with lead exposure were evaluated retrospectively. C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and complete blood count (CBC) were evaluated as inflammatory markers. Participants were categorized into 3 groups according to blood lead levels as < 10 µg/dL (Group 1), 10-30 µg/dL (Group 2), and > 30 µg/dL(Group 3). The association between NLR and lead was evaluated by inter-group and correlation analysis. Results: Median NLR values of Group 1, Group 2 and Group 3 were 1.45 (1.57), 1.90 (6.44), and 1.96 (6.36) respectively (p<0.001). NLR correlated positively with blood lead levels (r=0.412; p<0.001). A positive correlation was also detected with CRP, ESR, white blood cell (WBC), neutrophil, and mean platelet volume (MPV) levels (r=0.140; p<0.001, r=0.075; p=0.002, r=0.237; p<0.001, r=0.585; p<0.001, r=0.060; p<0.012, respectively). There was a negative correlation with lymphocyte (r= -0.536; p<0.001). Discussion: To our best knowledge, this study is the first one which shows a strong and dose-dependent association between NLR and lead levels.

Keywords

Lead; Neutrophil; Lymphocyte; Inflammation

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Introduction

As the toxicity of heavy metals to human health has become more evident, preventive measures against their exposure have been increasing over time. Risk thresholds are determined by measuring the levels of toxic and heavy metals in the air, underground and drinking water, and food. Then, preventive and/or therapeutic approaches are applied to reducelevels that may affect human health [1-2]. Although the effects of exposure to heavy metals and toxic chemicals have been effectively diminished in daily life, occupational exposure of workers in production and chemical industries continues to be an important health problem [3].

Lead is the most frequently encountered occupational and environmental heavy metal exposure in most countries. Studies have demonstrated nephrotoxic, hepatotoxic, neurotoxic, and genotoxic effects of heavy metals such as mercury, arsenic, cadmium, and especially lead [4-5]. Lead exposure is known to increase inflammatory response by causing oxidative stress through the production of reactive oxygen species (ROS) [6]. Exposure to an especially low dose of heavy metal for a long period is important because of inflammation formation, follow-up, and likely problems.

In routine practice, white blood cell (WBC) count, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) are the most commonly used inflammatory markers. Neutrophil lymphocyte ratio (NLR), which has been used in recent years, is a cheap and practical marker that can easily be calculated from complete blood count (CBC). NLR, used as a subclinical inflammatory marker, has been associated with the prognosis and mortality of many diseases [7-8]. In the context of this information, the objective of this study is to evaluate the association between NLR and levels of lead in workers with exposure to lead.

Material and Method

Study population

In this study, a retrospective evaluation of 4946 patients presenting to Ankara Occupational Diseases Hospital with lead exposure from January 2011 to March 2015 was done. In order to minimize possible effects, patients with the following diseases or conditions were excluded from the study: coronary artery disease (defined by patient history, physical examination, electrocardiogram, and laboratory results), diabetes mellitus, hypertension, hyperlipidemia, chronic obstructive pulmonary disease, acute or chronic inflammatory/infectious condition such as ankylosing spondylitis, rheumatoid arthritis, hepatitis, history of anti-inflammatory drug use (acetylsalycylic acid, corticosteroids, non-steroid anti-inflammatory drugs), smokers, patients with leukocytosis (>12000/µl) or leukopenia (<3500/µl) and abnormal renal, liver, or thyroid function tests. After taking into account these exclusion criteria, 3126 patients were not included in the study, and the study was carried out with a total of 1820 individuals.The greatest number ofindividuals were excluded because they were active smokers.

Of the 1820 participants, 527 (28.9%) were involved in battery production, 732 (40.2%) were involved in metal recycling, 173 (9.5%) were welders, 198 (10.9%) were smelters, and 190 (10.5%) were involved in metal production. All of the participants were workers with a 5 day-a-week and 8 hour-a-day schedule. Most of the participants' workplaces were small and medium-sized enterprises. Therefore, in most of these workplaces, measurements of environmental exposure/toxic substances were not done. In the small number of workplaces where measurements were done, accurate values could not be obtained because of incorrect measurements and inadequate data. This study was approved by the local ethics committee.

Collection of biological samples

Blood samples were taken from the participants at 'end of workshift.' Blood samples were drawn in 10 mm tubes with red caps not containing gel (BD Vacutainer) for serum CRP, AST, ALT, creatinine, and blood urea nitrogen analyses; in 10 mm EDTA-containing trace elements tubes (BD Vacutainer) for whole blood lead analysis, and in 5 mm EDTA-containing tubes (BD Vacutainer) for CBC and ESR analyses. For serum analyses, the specimens were centrifuged at 1500g for ten minutes after at least 30 minutes of incubation. All samples were analyzed on the same day.

Analysis methods

Serum creatinine levels were studied by Jaffe reaction, blood urea nitrogen levels were studied by enzymatic method, CRP levels were studied by latex-enhanced turbidimetric immunoassay method, and AST and ALT levels were studied by kinetic method with Konelab Prime 60 device (Thermo Scientific, Helsinki, Finland). CBC was analyzed on Beckman Coulter GenS instrument (Brea, CA, USA) and ESR analysis was performed by Alifax Test 1 (Padova, Italy). Whole blood lead levels were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Agilent 7700 series, Tokyo, Japan). Blood samples were digested by the microwave induced acid digestion method. Standard solution of lead was prepared by dilution of certified standard solutions (High-PurityStandards, Charleston, SC, USA). Two levels of quality control materials were used (Seronorm, Billingstad, Norway).The calibration curve of lead ranged from 0 to 100 µg/dL. Limit of detection and limit of quantification were 0.02 and 0.1 µg/dL, respectively. % Relative Standard Deviation of measurements was 4.2. Participants with lead exposure were categorized into three groups according to blood lead levels as < 10 µg/dL, 10-30 µg/dL and > 30 µg/dL [9].

Statistical Analysis

Statistical analysis of data was made by using the SPSS (Version 18.0) (SPSS Inc, Chicago, IL, USA) package program. Coherence to normal distribution analysis was made by using Kolmogorov-Smirnov test. Values were presented as mean ± SD or median (range). The presence of a statistically significant difference between the groups in terms of continuous variables was examined with ANOVA for parametric and Kruskal Wallis test for non-parametric variables. For the significant ($p<0.05$) analytes, Student's T test for parametric and Mann–Whitney U test for non-parametric variables were performed; Spearman's correlation analysis was also performed.

Results

The demographic and laboratory characteristics of 1820 male patients with lead exposure who participated in the study are

Table 1. Biochemical parameters and demographic characteristics of exposed groups

Parameters	Group 1 (n=697)	Group2 (n=692)	Group3 (n=431)	P
Lead (ug/dL)	2.90(9.80)	15.30(19.90)	44.5(2494)	<0.001 a,b,c
ALT (U/L)	23(276)	21(99)	19(262)	<0.001 a,b,c
AST(U/L)	20(99)	19(54)	20(157)	0.448
BUN (mg/dL)	13(46.90)	14(50)	14(45)	0.102
Creatinine (mg/dL)	0.89(1.38)	0.80(0.85)	0.81(1.22)	<0.001 a,b,c
CRP (mg/L)	1.37(55.77)	1.5(158.10)	1.5(9.99)	0.282
ESR (mm/hr)	2(47)	2(52)	2(20)	0.541
RBC (1012/L)	5.09(2.53)	5.15(2.72)	5.18(3.05)	<0.001 a,b
WBC (103/µL)	6.09(8.54)	7.35(7.70)	7.50(8.21)	<0.001 a,b
HGB (g/dL)	15.40(7.80)	15.60(9.40)	15.20(9.30)	<0.001 a,b,c
Hematocrit %	45.10(22.80)	45.20(24.90)	45(26.10)	0.098
Platelets (103/µL)	223(362)	222(343)	218(290)	0.599
Neutrophils (109/L)	3.60(6.10)	4.40(6.70)	4.40(6.90)	<0.001 a,b
Lymphocytes (109/L)	2.5(4.20)	2.20(5)	2.20(3.40)	<0.001 a,b
NLR	1.45(1.57)	1.90(6.44)	1.96(6.36)	<0.001 a,b
MPV(fL)	8.30(7.40)	8.40(5.90)	8.40(5.60)	0.453
Age (year)	37.5 ± 8.6	37.4 ± 8.8	35.5 ± 8.8	0.267
Working duration (year)	14.8 ± 8.2	18.4±6.9	15.1±6.3	0.125

a: significant difference between Group 1 and Group 2, b: significant difference between Group 1 and Group 3, c: significant difference between Group 2 and Group 3, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, BUN: Blood urea nitrogen, CRP: C-reactive protein, ESR: Sedimentation, RBC: Red blood cells, WBC: White blood cells, HGB: Hemoglobin, NLR: Neutrophil lymphocyte ratio, MPV: Mean platelet volume

demonstrated in Table 1 .No significant difference was observed between the groups in terms of age or working duration. Median NLR values of Group 1, Group 2, and Group 3 were 1.45 (1.57), 1.90(6.44), and 1.96 (6.36) respectively (p<0.001) (figure 1). Analysis using Spearman's correlation coefficient showed that NLR correlated positively with blood lead levels (r=0.399; p<0.001; figure 2). A positive correlation was also detected with CRP, ESR, WBC, neutrophil, and MPV levels (r=0.140, p=0.000; r=0.075, p=0.002; r=0.237, p=0.000; r=0.585, p=0.000; r=0.06, p=0.012).There was a negative correlation between NLR and levels of lymphocyte (r=-0.536, p<0.001). There was no correlation between ALT, AST, blood urea nitrogen, creatinine, RBC, HGB, HCT, and PLT (r= -0.021, p=0.372; r=0.040, p=0.372; r= -0.037 p=0.149; r= -0,045, p=0.057; r=0,045, p=0.059; r=0,044, p=0.064; r=0.00, p=0.984; r= -0.046, p=0.055).

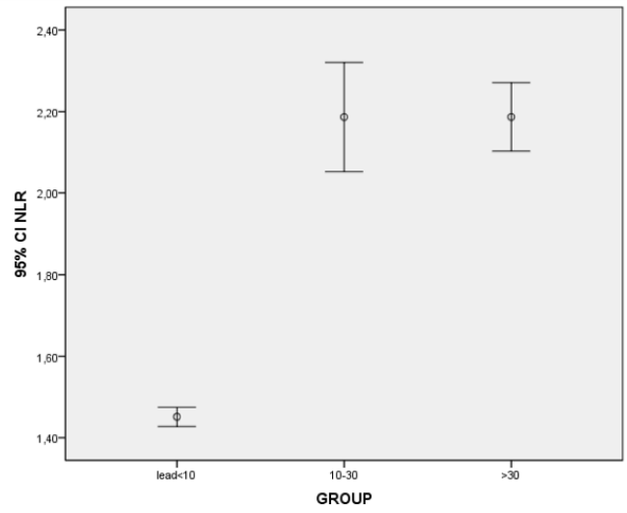

Figure 1. Boxplot demonstrated that neutrophil lymphocyte ratiohas been found to be higher in patients with high blood lead levels.

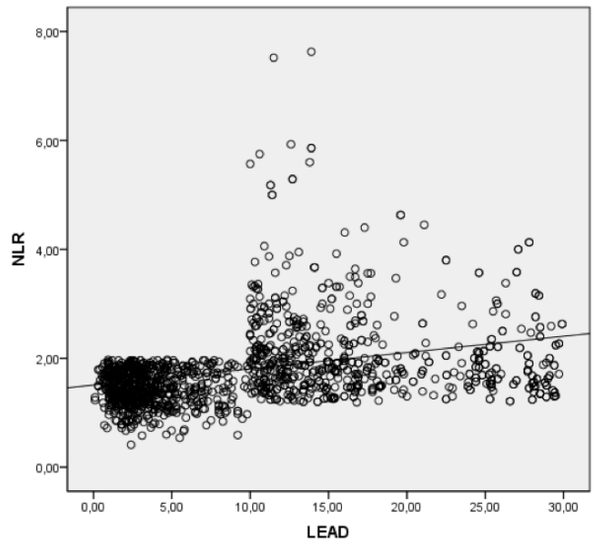

Figure 2. Correlation of neutrophil lymphocyte ratioand lead levels (0-30ug/dL), (r=0.399, p<0.001)

Discussion

Follow-up of inflammation is most frequently detected by analysis of CRP, sedimentation, and WBC count. In recent years, NLR, a marker showing the inflammatory status of the body and calculated from blood neutrophil and lymphocyte counts, has been investigated as an alternative and promising marker. To the best of our knowledge, this is the first time that there is a significant association between lead exposure and NLR which has been found to be higher in patients with high levels of blood lead. Moreover, a positive and strong correlation has also been demonstrated between levels of blood lead and NLR.

Like many other toxic metals, lead also causes cellular changes through oxidative stress. Besides, the pathogenesis of lead intoxication is multifactorial and in addition to oxidative stress, inflammation also plays a role. There is a very strong relationship between oxidative stress and inflammation. Oxidative stress stimulates apoptosis and inflammation by increasing the production of nuclear factor (NF-KB) [10]. On the other hand, reactive oxygen species (ROS) secreted from immune cells activated by inflammation stimulates oxidative stress. Therefore, oxidative stress and inflammation continuously stimulate each other resulting in a vicious circle [11].

In many studies, it has been demonstrated that lead stimulates oxidative stress by increasing the production of ROS [12].The ROS thus formed initiates an inflammatory process by causing damage to cell membranes through lipid peroxidation [13].On the one hand, lead causes tissue damage and inflammation by increasing ROS through various pathways. On the other hand, the precipitated inflammation increases oxidative damage by positive feedback and potentiates the negative effects of lead. As a result, it is thought that inflammation and oxidative stress are responsible for the negative effects of lead [14].

Another important effect of lead on the human body is seen

through glutathione metabolism. Many heavy metals, particularly lead, show high affinity with sulfhydryl complex bound to glutathione. Glutathione reductase, glutathione peroxidase, and glutathione S transferase, enzymes that are important in glutathione metabolism and that also exhibit antioxidant properties, are deactivated by lead and an oxidative environment is formed [15].Glutathione is a protein rich in cysteine andmost of it is produced in lymphocytes (>90%) [16].Therefore, after exposure to lead, counts of lymphocyte decrease and so does the production of an important antioxidant, glutathione. Along with direct effects of lead on glutathione metabolism, this lymphocyte-mediated decrease further nullifies antioxidant property.

In a study performed by Kim et al., TNF-alpha and WBC counts were found to be higher in individuals with lead exposure when compared to those of the control group [18].Again, in the same study, a significant correlation was found between blood lead levels and these inflammatory markers. Also, in other studies, a similarlysignificant correlation has been demonstrated between levels of blood lead and another important inflammatory marker, hs-CRP [15-18].This study also demonstrated similar results with a significant correlation found between blood lead levels and ESR, CRP, and WBC counts.

In this study, NLR was found to increase with a rise in lead levels with absolute WBC and neutrophil counts rising and absolute lymphocyte counts falling. Results similar to the present study have been demonstrated in experimental studies performed on guinea pigs. Guinea pigs were randomized as controls and lead-exposed and they were evaluated in terms of different inflammatory markers. In the lead-exposed group, total protein, WBC count, and phospholipase A2 levels were found to be significantly higher when compared with the control group. When WBC subgroups were evaluated, neutrophil, basophil, and eosonophil ratio (WBC%) were found to be significantly higher while lymphocyte ratio (WBC%) was found to be significantly lower in the lead-exposed group when compared to the control group [19].

Leukocytes and especially neutrophils are key cellular components in inflammation and they each play a role by secreting different cytokines that govern the production of ROS and toxic mediators. During inflammation, increased glucocorticoid levels and increased apoptosis in response to stress cause lymphocyte counts to decrease [20].However, information on glucocorticoid levels during lead exposure is very limited. Lead has been demonstrated to increase glucocorticoid levels in animal experiments [21-22]. On the other hand, studies performed in humans have demonstrated contradictory results [23-24]. The effect of glucocorticoid on decrease of the lymphocyte count during lead exposure is controversial. Therefore, the effect of lead on the hypothalamus-hypophysis-adrenal axis needs to be investigated further. In addition to inflammatory, stress, and hormonal factors, direct toxicity of lead on the immune system also plays a role in the pathophysiology of lymphocytopenia. Lead not only affects proliferation, but also differentiation of lymphocytes through receptors, intracellular pathways, and DNA [25].

Lead exerts its toxicity through oxidative stress, cytotoxicity, and genotoxicity; however, chronic and systemic inflammation also play a pivotal role. This study has for the first time demonstrated that there is a strong and dose-dependent association between NLR, which is a simple, cheap, easily available, and reproducible marker of inflammation, and lead. Larger experimental and mechanistic studies are needed to understand the pathogenesis of lead toxicity and to develop specific therapeutic pathways.

Competing interests

The authors declare that they have no competing interests.

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